



How many genera and species of woolly monkeys (Atelidae, Platyrrhine, Primates) are there? The first molecular analysis of *Lagothrix flavicauda*, an endemic Peruvian primate species



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ABSTRACT

We sequenced *COI* and *COII* mitochondrial genes of 141 Neotropical woolly monkeys to provide new insights concerning their phylogeography and phylogenetic relationships. For the first time, eight individuals of the endemic and extremely rare Peruvian yellow-tailed woolly monkey (*flavicauda*) were sequenced at these genes and compared with other *Lagothrix* taxa (*poepigii*, *lagotricha*, *lugens* and *cana*). There were four main results. (1) *L. flavicauda* showed a gene diversity of zero, whereas *poepigii* and *lugens* showed high levels of gene diversity and *lagotricha* and *cana* showed more modest levels of gene diversity. The absence of gene diversity found for *L. flavicauda* strongly supports that it is one of the 25 more endangered primates on earth; (2) Our genetic distance and phylogenetic analyses, which included many cases of genetic introgression and recent hybridization, suggest that all woolly monkeys could be included in one unique genus, *Lagothrix*, divided into two species: *L. flavicauda* and *L. lagotricha*. The last species is divided into at least four subspecies. Our molecular results agree with Fooden's (1963) classification, but do not support the classification proposed by Groves (2001). (3) *Poepigii* was the first taxon within *L. lagotricha* to experience a mitochondrial haplotype diversification, while *cana* and *lagotricha* experienced more recent mitochondrial haplotype diversification; (4) *Poepigii* and *lagotricha* were the taxa which showed the greatest evidence of population expansions in different Pleistocene periods, whereas *lugens* experienced a population decline in the last 25,000 YA.

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1. Introduction

Lagothrix (E. Geoffroy, 1812) is one of four Neotropical Primate genera of the Atelidae family—containing the largest living primates in Latin America. These primates have distinct thick woolly fur (woolly monkeys), strong prehensile tails and occupy a critically important niche linked to the successful dispersal and recruitment of trees (Levi and Peres, 2013). Thus, their conservation is of prime concern, not just for sake of the woolly monkeys themselves, but also for the preservation of Amazonian forests. Rightly so, conservation biology depends on the identification and description of species and agreed upon taxonomic categories. However, unfortunately, there is general disagreement among primatologists over the taxonomy of *Lagothrix*. It is our intention to resolve this debate by the presentation of new molecular findings.

Here, we begin with a brief review of the literature regarding taxonomic characters of *Lagothrix*. A more detailed review along with references are provided in Table 1. Fooden (1963) determined that this genus was integrated by two species: *L. flavicauda* (yellow-tailed woolly monkey), an endemic species in Peru, and *L. lagotricha* (Humboldt's woolly monkey), distributed in Colombia, Venezuela, Ecuador, Peru, Brazil and recently discovered in Bolivia (Wallace and Painter, 1999).

Ever since Alexander von Humboldt provided the first description of the yellow-tailed woolly monkey in 1812, there have been consistent changes in its taxonomy. During this time it has been redefined, placed together with *Ateles*, separated from *L. lagotricha*, and even considered as a full genus (Table 1). These variations in taxonomy may be due to the lack of molecular data needed to help determine the relationship of *flavicauda* with other *Lagothrix* and Atelidae taxa.

Taxonomic questions of this species are further complicated because of the difficulty in finding samples. It has a very limited

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Table 1

Chronology of taxonomy regarding the yellow-tailed woolly monkey.

Date	Author(s)	Summary
1812	von Humboldt	Analyzed skins and determined yellow-tailed woolly monkey to be <i>Simia flavicauda</i>
1927(a,b)	Thomas	Redefined the yellow-tailed woolly monkeys as <i>Oreonax hendeei</i>
1963	Fooden	Combined <i>Simia flavicauda</i> and <i>Oreonax hendeei</i> into a single species called <i>Lagothrix flavicauda</i>
2001	Groves	Based on an examination of skulls from museums, <i>Lagothrix flavicauda</i> is grouped with <i>Ateles</i> and considered separate from <i>Lagothrix lagotricha</i> . <i>Flavicauda</i> was considered a full genus called <i>Oreonax flavicauda</i>
2008(a,b)	Rosenberger	<i>Oreonax</i> is not different from <i>Lagothrix</i> . The yellow-tailed woolly monkey is renamed as <i>Lagothrix flavicauda</i>

geographic distribution in the Peruvian premontane, montane and cloud forests between 1500 and 3000 m above sea level (masl). Many of these areas are inaccessible and the species has an extremely small population size. The species probably has a distribution in the Peruvian Departments of Amazonas, San Martín, Huánuco, Loreto and La Libertad as predicted by an ecological model (Pacheco et al., 2007). However, the species presence has only been verified in two Peruvian Departments, Amazonas and San Martín (DeLuycker, 2007; Shanee et al., 2008). Leo Luna (1989) did report nine locations where this species was present in the 1980s; however, its presence could not be demonstrated in other areas where this species was previously reported during the 1970s and 1980s (Pedro Ruiz Gallo; Yamborasbamba, Gira-Sisa Reserve, Shimbayacu). More recently, DeLuycker (2007) and Shanee et al. (2008) showed a detailed study where this taxon is distributed (Table 2).

No thorough censuses have been carried out of this taxon, but Nowak (1999) estimated that there were less than 250 individuals (although this number could be somewhat greater) in the wild.

Also, there are no individuals of this species found in any of the world's public zoos. This species is listed as Critically Endangered by IUCN in 2014 and it is classified as one of the 25 most endangered primate taxa (Mittermeier et al., 2007).

Humboldt's Woolly monkey (*Lagothrix lagotricha*) has also undergone taxonomic changes since its first description by Humboldt in 1812. Its taxonomy is based on Fooden's analysis of 312 specimens (Fooden, 1963). He identified four different subspecies within *Lagothrix lagotricha*: *L. l. lugens*, *L. l. lagotricha*, *L. l. poeppigii*, and *L. l. cana*. Their distributions and phenotypes are described in Table 3. Groves (2001), however, elevated these *L. lagotricha* taxa to the status of different species. Thus, this author considered the existence of two woolly monkey genera, *Oreonax*, with a unique species, *O. flavicauda*, and *Lagothrix* with four species: *L. lugens*, *L. lagotricha*, *L. poeppigii* and *L. cana*.

Only two molecular studies have been published to help resolve the systematics questions regarding the woolly monkeys (Botero et al., 2010; Ruiz-García and Pinedo 2010).

Botero et al. (2010) analyzed 16 samples collected from Colombian zoos. Unfortunately, as often occurs with these kind of samples, the authors could not determine the exact geographical origins of their exemplars. Additionally, only phenotype individuals belonging to *lugens* and *lagotricha* were enclosed in their study. The authors sequenced two mitochondrial genes (*mtCOII* and the *mt D-loop*), analyzed the karyotypes, and provided three main and important conclusions. They determined that there were no significant differences in the variation of karyotype frequency at chromosomes 4, 7 and 24 between *lugens* and *lagotricha*. This finding supported a subspecies status rather than a species status for these *Lagothrix* taxa. They obtained a similar conclusion when they constructed and analyzed a Bayesian tree with *lugens* and *lagotricha* haplotypes intermixed. The authors also detected a very recent split between *lugens* and *lagotricha* at the beginning of the Holocene.

Ruiz-García and Pinedo (2010), analyzed the mitochondrial *COII* gene (*mtCOII*) of 97 *Lagothrix lagotricha* specimens, belonging to the

Table 2

Geographic distribution of yellow-tailed woolly monkey in Peru.

Distribution	References
• Nine locations within two Peruvian Departments 1. Between Pongo de Rentema and Bagua 2. Two points in the Ulcubamba River 3. Two points between the Chiriago and Mayo Rivers 4. One point west of Chachapoyas 5. One point between Rioja and Mendoza 6. Two points in the Southwestern area of the San Martín 7. Department Frontier with La Libertad near Achiras and Paulina	Leo Luna (1989)
Wide areas • Three or four regions within two Peruvian Departments (Amazon and San Martín Departments) 1. Colan Cordillera (641 km²) 2. Upper Mayo River (1820 km²) 3. Abiseo River National Park (2740 km²) 4. Maybe, Laguna de los Cóndores	DeLuycker (2007)
Precise areas • Five locations within the Amazon Department 1. Santa Rosa 2. Shipasbamba 3. Abra Patricia 4. Gocta 5. La Perla de Limasa • Three locations within the Martín Department 1. Paitoja 2. Colca 3. Nuevo Mendoza	Shanee et al. (2008) Shanee et al. (2008)

lugens, *lagotricha*, *poeppigii* and *cana* taxa. This study produced several important findings. *Poeppigii* and *lugens* showed the highest levels of gene diversity, whereas *lagotricha* and *cana* showed the lowest. The authors also suggested that an ancestor of *poeppigii* was a possible candidate for the beginning of the diversification of *L. lagotricha*. They also detected different mitochondrial lineages within *poeppigii* and *lugens* and even hybrid individuals between *lagotricha* and *lugens*, between *lagotricha* and *poeppigii* and between *lugens* and *poeppigii*. Also, all of their samples came from the wild and hybridization was considered to be natural among these taxa. Such an interpretation suggests that these taxa are subspecies and not full species as claimed by Groves (2001). The beginning of the mitochondrial haplotype diversification in *L. lagotricha* began around 2.5 MYA—coinciding with the beginning of the Pleistocene. Lastly, the authors' data supports haplotype diversification within *poeppigii* and *lugens* during the first and second Pleistocene glacial periods and the diversification of *lagotricha* and *cana* during the third and fourth Pleistocene glaciations. Their results agree quite well with the results reported in the previous mentioned work of Botero et al. (2010). That is, both taxa are subspecies and not full species such as suggested by Groves (2001).

In this study, we sequenced two mitochondrial genes, the cytochrome c oxidase subunits I and II (*mt COI* and *COII*). The

Table 3

Distributions and phenotypes of subspecies of Humboldt's woolly monkey.

Name	Phenotype	Distribution
<i>L. l. lugens</i>	Silvery grey to black; darkness of coat and hair length are positively correlated with elevation	Eastern slope of the Eastern Colombian Cordillera northward to the Guayabero and Apuré Rivers near the border between Colombian and Venezuela. Fragmented populations are distributed in the region of the upper Magdalena R. (especially the Huila and Tolima Dept.) and in the Central Cordillera along the course of the Magdalena R. (Serranía de San Lucas in the Bolívar Dept, upper San Jorge Valley, in the Córdoba Dept and in the Antioquia and Santander Dept). Elevation range: 100–3000 m above sea level (masl)
<i>L. l. lagotricha</i>	Uniformly pale brown color; pale-colored crown	North of the Amazon R. from the north bank of the Napo-Aguarico rivers in Ecuador and Peru to the west side of the Río Negro in Brazil, to the upper Orinoco in Venezuela, including the Colombian Amazon to the Guaviare R. in Southern Vichada. Elevation range: sea level to 400 masl
<i>L. l. poeppigii</i>	Reddish brown; dark-brown crown	South side of the Napo and Aguarico rivers across a large fraction of the Loreto Dept. in the Peruvian Amazon, including the Marañón and Ucayali Rivers, and the Ecuadorian Amazon to the Jurúa R. in the Brazilian Amazon. Elevation range: sea level to 800 masl (up to 1200 m in parts of the Ecuadorian and Peruvian Andes)
<i>L. l. cana</i>	Bluish grey or olivaceous body Color with brown-blackish head; dark hands, feet, and tail; no mid-sagittal streak on tail	East side of the Jurúa R. to the west side of the Tapajós R., mainly in the Brazilian Amazon; the Madre de Dios River in Peru; isolated patches in Northern Bolivia as well as northward along the left bank of the upper Ucayali R. to the lower Pachitea R. Elevation range: sea level to 1600 masl in the Andes near Cuzco

mitochondrial genes are interesting markers for phylogenetic tasks because they include a rapid accumulation of mutations, lack introns, have a high number of copies per cell and a negligible recombination rate, and include haploid inheritance (Avice et al., 1987). The selected genes are two of the three mitochondrial DNA encoded subunits (*COI*, *COII* and *COIII*) of respiratory complex IV. This complex is the third and final enzyme of the electron transport chain of mitochondrial oxidative phosphorylation (Capaldi, 1990).

The first gene has emerged as the standard barcode region for animals, including mammals (www.mammaliabol.org). Hebert et al. (2003a,b, 2004a,b) have strongly argued in favor of using a 5' fragment of this mitochondrial gene as a barcoding marker that has shown to provide a sufficient resolution and robustness in some groups of organisms, such as arthropods, fishes, birds and mammals to distinguish species (Agrizzi et al., 2012; Borisenko et al., 2008; Elmeer et al., 2012; Lim, 2012).

The second gene has been studied extensively in primate phylogenetics: within the Hominoidea (Adkins and Honeycutt, 1991; Ruvolet et al., 1991), within the Cercopithecoidea (Disotell et al., 1992), within the Strepsirrhini (Adkins and Honeycutt, 1994) and also in several neotropical Primate genera and species, such as *Aotus* (Ashley and Vaughn, 1995; Plautz et al., 2009; Ruiz-García et al., 2011, 2013a), *Alouatta* (Figueiredo et al., 1998; Cortes-Ortiz et al., 2003; Ruiz-García et al., 2013b), *Ateles* (Collins and Dubach, 2000a,b; Ruiz-García et al., 2013c), *Cebus apella* (Ruiz-García et al., 2012a), *Cebus albifrons* (Ruiz-García et al., 2010), *Cebus capucinus* (Ruiz-García et al., 2012b), *Saimiri* (Ruiz-García et al., 2013d), *Saguinus leucopus* (Ruiz-García et al., 2013e) and other Callitrichidae (Sena et al., 2002). Adkins and Honeycutt (1994) and Andrews and Eastale (2000) demonstrated that monkeys and apes have undergone a two to threefold increase in the rate of amino acid replacement (nonsynonymous substitutions) with regard to other primates and other mammals and that the evolutionary rate of this gene is basically the same (in monkeys and apes) to that reported for *mtCyt-b* and *mtCOI*. These three genes may be part of a multi-enzyme evolutionary phenomenon and therefore are equally affected. Asuncion et al. (2003) also showed that a phylogenetic signal (g_1) was present in all codon positions of this gene, despite having transitional saturation at the third position. Also, the bootstrap support values were very high below the genus level but abruptly decreased above this level. The authors concluded that this marker could be very useful in molecular platyrrhine systematics, both at intra-generic and at intra-specific levels.

Therefore, this is the third molecular phylogenetic and phylogeographic study on the woolly monkeys including for first time, the extremely endangered endemic Peruvian yellow-tailed woolly monkey. Furthermore, this study contains the largest sample size

of woolly monkeys analyzed to date (141 individuals) and for the first time contains the results of *mtCOI* sequences for this Neotropical primate group.

This study (focusing on *mtCOI-COII* genes) was initiated to meet four main objectives. (1) To determine the number of subspecies, species and genera of woolly monkeys detectable by these mitochondrial genes—using levels of genetic heterogeneity, genetic distances and different phylogenetic procedures; (2) To determine the levels of gene diversity within the different taxa analyzed and to relate them with the antiquity of these taxa as well as with their conservation status; (3) To estimate the possible temporal splits among these woolly monkey taxa and to correlate them with climatic and geological changes and (4) To determine possible historical demographic changes throughout the natural history of these taxa.

2. Materials and methods

2.1. Samples

A total of 141 woolly monkey individuals were sequenced for two mitochondrial genes. These 141 individuals were phenotypically and geographically classified. There were eight individuals of *L. flavicauda*, 26 individuals of *L. l. lugens*, 30 specimens of *L. l. lagotricha*, 47 exemplars of *L. l. poeppigii* and 30 individuals of *L. l. cana*. The outgroups contained five *Alouatta seniculus* (one from Leticia, Amazon Department, Colombia, three from Requena, Tapiche River, Peru and one from the Nanay River, Peru), one *Ateles chamek* (from Mamore River, Bolivia) and one *Aotus azarae boliviensis* (from the Santa Cruz Department, Bolivia). Also, a sample of *Brachyteles hypoxanthus* (from Brazil) was enclosed in the Bayesian tree. The geographical origins of these animals can be observed in Table 4.

The DNA of all the individuals of *L. flavicauda*, *L. l. lugens* and *L. l. cana* and a fraction of the *L. l. lagotricha* and *L. l. poeppigii* was extracted from hairs obtained from animals found alive in diverse Indian and colonos communities throughout Colombia, Peru, Ecuador and Brazil. Another fraction of the DNA was obtained from the tissues of hunted *L. l. lagotricha* and *L. l. poeppigii*. We requested permission to collect biological materials from these hunted animals that were already present in the Indian communities. In these cases we sampled small pieces of muscles or teeth. Communities were visited only once. All sample donations were voluntary, and no financial or other incentive was offered for supplying specimens for analysis. All the pets and the hunted animals analyzed were obtained by the Indian communities at a maximum of 20 km from their community. For more information about sample permissions, see the Acknowledgment section.

2.2. Molecular analyses

2.2.1. *mtCOI* and *COII* Genes

The DNA from muscle was extracted using the phenol-chloroform procedure (Sambrook et al., 1989), whereas DNA samples from hair and teeth were extracted with 10% Chelex resin (Walsh et al., 1991). For the *mtCOI* amplification (polymerase chain reaction, PCR), we used the forward primer LCO1490 (5'-GGTCAA-CAAATCATAAAGATATTGG-3'), and the reverse primer HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (657 base pairs, bp) (Folmer et al., 1994) under the following PCR profile: 94 °C for 5 min, followed by 39 cycles of 94 °C for 30 s, 44 °C for 45 s, 72 °C for 45 s, and a final cycle of 72 °C for 5 min. For the amplification of the *mtCOII* gene (located in the lysine and asparagine tRNAs) we used the forward primer L6955 (5-AACCATTTCATAACTTTGTCAA-3) and the reverse primer H7766 (5-CTCTTAATCTTAACT-TAAAAG-3) (720 bp) (Ashley and Vaughn, 1995; Collins and Dubach, 2000a; Ruiz-García et al., 2010, 2012a, 2012b). We used the following temperatures: 95 °C for 5 min, 35 cycles of 45 s at 95 °C, 30 s at 50 °C and 30 s at 72 °C and a final extension time for 5 min at 72 °C. For both genes, the PCRs were performed in a 25 µl volume with reaction mixtures including 4 µl of 10 × buffer, 6 µl of 3 mM MgCl₂, 2 µl of 1 mM dNTPs, 2 µl (8 pmol) of each primer, 2 units of Taq DNA polymerase, 5 µl of H₂O and 2 µl (20–80 ng/µl) of DNA. PCR reactions were carried out in a BioRad thermocycler. All amplifications, including positive and negative controls, were checked in 2% agarose gels, using the molecular weight marker φ 174 DNA digested with *Hind* III and *Hinf* I. The amplified samples were purified using membrane-binding spin columns (Qiagen). The double-stranded DNA was directly sequenced in a 377A (ABI) automated DNA sequencer. The samples were sequenced in both directions using the BigDye TM kit and all the samples were repeated to ensure sequence accuracy. The sequences were deposited in GenBank and some of the specimens had previously been analyzed at *mtCOII* (Ruiz-García and Pinedo 2010). However, there are two important differences between the previous and current papers. The sample size increased and, for the first time, the *mtCOI* gene was sequenced for all sampled specimens. As the population genetics and phylogenetic analyses of both mitochondrial genes support similar findings, we only highlight the results of when both genes were analyzed together (657 bp + 720 bp = 1377 bp).

It is possible that some of the sequences represent numts (mitochondrial DNA fragments inserted into the nuclear genome) rather than true mtDNA (Chung and Steiper, 2008). However, we note that all amino acid translations of the obtained sequences showed the presence of initial start and terminal stop codons and the absence of premature stop codons. Protein translation was also checked to evaluate the possible presence of numts. Nevertheless, the mutations we observed were synonymous changes, thus suggesting that there were no numts in the sequences. Moreover, one would expect to see a signal relating to numts in the DNA chromatogram. When the ratio of nuclear to mitochondrial genomes per cell is around 1/1000 (Takamatsu et al., 2002), the proportion of numts and real mitochondrial sequences is expected to be amplified. If the potential amplified numts were highly differentiated from the real mitochondrial gene or included some insertions or deletions, many double peaks are expected in the chromatogram. We did not observe this in our chromatograms. Nevertheless, the possibility of numt amplifications can never be completely discarded, and this must be taken into consideration when interpreting the results.

2.3. Data analyses

2.3.1. Genetic diversity and heterogeneity analyses

The statistics used to determine the genetic diversity within the diverse woolly monkey groups were as follows: the haplotypic

diversity (H_d), the nucleotide diversity (π), the average number of nucleotide differences (k) and the θ statistic by sequence. Several procedures were carried out to estimate the genetic heterogeneity among the diverse *Lagothrix* taxa analyzed. To determine the genetic heterogeneity within the *Lagothrix* genus, one procedure was applied to the haplotypic frequencies (G_{ST} statistic; Nei, 1973) and other genetic heterogeneity statistics were applied to the nucleotide sequences (γ_{ST} , N_{ST} and F_{ST} statistics, Hudson et al., 1992). These gene diversity and genetic heterogeneity statistics were undertaken with the programs DNAsp 5.1 (Librado and Rozas, 2009) and Arlequin 3.5.1.2 (Excoffier and Lischer, 2010).

2.3.2. Phylogenetic analyses

The sequence alignments were carried out manually as well as with the DNA Alignment program (Fluxus Technology Ltd.). Modeltest (Posada and Crandall, 1998) and Mega 5.1 (Tamura et al., 2011) were applied to determine the best evolutionary mutation model for the 141 sequences analyzed. We used the Akaike Information Criteria (AIC; Akaike, 1974) to determine the best evolutionary mutation model.

We obtained maximum likelihood estimates of transition/transversion bias as well as maximum likelihood estimates of the gamma parameter for site rates of the best evolutionary mutation model (Tamura et al., 2011).

We constructed phylogenetic trees using four different procedures. For example, we used the Neighbor-joining (NJ; Saitou and Nei, 1987) tree with the Tamura 3P genetic distance (Tamura, 1992) and the Maximum likelihood (ML; Felsenstein, 1981) tree with the GTR model. We also relied on the Maximum Parsimony (MP) tree (with close-neighbor-interchange with search level 1 and random addition with 100 replicates and with heuristic search with stepwise addition and branch swapping). All the trees were constructed with the PAUP*4.0b8 program (Swofford, 2002) and MEGA 5.1. Finally, used a Bayesian tree (BT; Mau, 1996; Mau et al., 1999; Rannala and Yang, 1996) developed with a GTR model of nucleotide substitution with the gamma distributed rate varying among sites, and 7 rate categories (GTR + G). This was determined to be the better model using the Modeltest program. This Bayesian analysis was completed with the BEAST v1.4.8 program (Drummond and Rambaut, 2007). Two separate sets of analyses were run, assuming a Yule speciation model and a relaxed molecular clock with an uncorrelated log-normal rate of distribution (Drummond et al., 2006). Results from the two independent runs (40,000,000 generations with the first 4,000,000 discarded as burn in and parameter values sampled every 1000 generations) were combined with LogCombiner v1.6.2 software (Drummond and Rambaut, 2007). Posterior probability values provide an assessment of the degree of support of each node on the tree. The final tree was estimated using TreeAnnotator v1.6.2 software (Drummond and Rambaut, 2007) and visualized in the FigTree v1.3.1 program (Drummond and Rambaut, 2007).

We used the Software Network 4.6.10 (Fluxus Technology Ltd.) to form a median joining network to estimate possible divergence times among the haplotypes (joined *mtCOI* and *COII* sequences) in woolly monkeys (Bandelt et al., 1999). The ρ statistic (Morrall et al., 1994) was estimated and transformed into years. To determine the temporal splits, it is necessary to estimate the mutation rate at these *mt CO* genes. Ruvolo et al. (1991) determined a mutation rate of 0.85% per million years per lineage for Hominoidea at *mt COII*. This represents 1 mutation on average each 199,402 years. This mutation rate was practically identical to that determined by Ruiz-García and Pinedo (2010) in the previous *Lagothrix* study (1 mutation on average every 191,000 years). Similarly, for *Aotus*, Ashley and Vaughn (1995) and Ruiz-García et al. (2011) determined 1 mutation on average every 199,000 years at this same mitochondrial gene. For *mtCOI*, an average mutation rate of

Table 4

Geographical origins of the 141 individuals of the *Lagothrix* genus sequenced at the *mt* *COI* and *COII* genes. Sample sizes analyzed = *n*. It is interesting to note that, for instance, the H7 was shared by *lugens*, *lagotricha* and *poepigii*.

Morphological species and subspecies of <i>Lagothrix</i> analyzed in this study following the classification of Fooden (1963)	Molecular lineage	Geographical origins	Haplotypes found in each molecular lineage	Cases of possible hybridization or genetic introgression
<i>Lagothrix flavicauda</i> (<i>n</i> = 8)	<i>flavicauda</i>	Peru Abra Patricia and Colca, Upper Mayo River (<i>n</i> = 2); Ulcabamba River (Bagua Grande and La Higuera) (<i>n</i> = 2); Santa Rosa (<i>n</i> = 1); Shipasbamba (<i>n</i> = 1); Paitoja (<i>n</i> = 1); Cordillera Colan (<i>n</i> = 1); all these points in the Peruvian Amazonas and San Martin Departments	H59	No cases found
<i>Lagothrix lagotricha lugens</i> (<i>n</i> = 26)	<i>lugens</i>	Colombia North Eastern Antioquia Department (<i>n</i> = 1); Southern Antioquia Department (<i>n</i> = 9); South Eastern Bolivar Department (<i>n</i> = 1); Magdalena Valley in Cordoba Department (<i>n</i> = 1); Cundinamarca Department (<i>n</i> = 5); La Macarena, Meta Department (<i>n</i> = 1); Huila-Tolima Departments (<i>n</i> = 6); Cauca Department (<i>n</i> = 1); Los Picachos National Park in Caqueta Department (<i>n</i> = 1)	H1, H2, H3, H4, H5, H6, H7, H19, H20, H21, H24, H25, H30, H32, H33, H55	Five cases of genetic introgression within <i>lugens</i> : two cases of <i>lagotricha</i> and three cases of <i>poepigii</i>
<i>Lagothrix lagotricha</i> (<i>n</i> = 30)	<i>lagotricha</i>	Colombia Nariño Department, Putumayo River (<i>n</i> = 1); Amazon Department (Cotubé River; El Vergel, Nuevo Jordan, Miriti-Paraná River, Apaporis River; Veinte de Julio-Loreto-Yaku River, Calderon, Putumayo River; Amacayacu National Park; El Porvenir, La Libertad, El Progreso) (<i>n</i> = 17); Caqueta Department (La Pedrera, Araracuara) (<i>n</i> = 2); Vaupes Department (Puerto Ibacaba) (<i>n</i> = 1); Guainia Department (Puerto Inirida) (<i>n</i> = 1) Ecuador Cuyabeno (<i>n</i> = 1) Peru Loreto Department (Nueva Unión and Santa Clotilde- Napo River; Pebas-Amazon River) (<i>n</i> = 3) Brazil Western bank of the Negro River (<i>n</i> = 4)	H7, H8, H14, H15, H17, H21, H22, H23, H26, H27, H37, H38, H39, H42, H65, H66	One case of genetic introgression of <i>lugens</i> within <i>lagotricha</i> . One case of recent hybridization between <i>lagotricha</i> and <i>lugens</i>
<i>Lagothrix lagotricha poeppigii</i> (<i>n</i> = 47)	<i>poeppigii</i>	Ecuador Nuevo Rocafuerte-Napo River (<i>n</i> = 1); Yasuni National Park-Napo River (<i>n</i> = 4); Pastaza Province (Puyo) (<i>n</i> = 9); Napo Province(Tena, Pano River) (<i>n</i> = 2) Peru Loreto Department (Sta. Maria-Napo River (<i>n</i> = 2); Chimbote-Amazon River (<i>n</i> = 1); Nanay River (<i>n</i> = 1); Caballococha-Amazon River (<i>n</i> = 1); Santa Rosa-Yavari River (<i>n</i> = 1); Requena-Tapiche River (<i>n</i> = 8)); San Martin Department (Yurimaguas-Huallaga River (<i>n</i> = 4); Moyobamba (<i>n</i> = 3); Tarapoto and Venecia Lake (<i>n</i> = 3)); Ucayali Department (Apagea-Alto Amazonas (<i>n</i> = 1); Yarinacocha (<i>n</i> = 1); Pisiqiu (<i>n</i> = 3)) Brazil Yavari River (Benjamin and Tecohavi) (<i>n</i> = 2)	H3, H7, H12, H13, H16, H18, H29, H31, H34, H36, H40, H41, H44, H45, H46, H47, H48, H49, H50, H51, H52, H53, H54, H55, H56, H58, H67, H68, H69, H70, H71, H72, H73, H74, H75, H76, H78, H79, H80, H81	Two cases of genetic introgression of <i>lugens</i> within <i>poeppigii</i> and two cases of recent hybridization between <i>poeppigii</i> and <i>lagotricha</i>

(continued on next page)

Table 4 (continued)

Morphological species and subspecies of <i>Lagothrix</i> analyzed in this study following the classification of Fooden (1963)	Molecular lineage	Geographical origins	Haplotypes found in each molecular lineage	Cases of possible hybridization or genetic introgression
<i>Lagothrix lagotricha cana</i> (n = 30)	<i>cana</i>	Brazil Purus River (n = 4); Tefé River (n = 8); Rio Branco-Acre (n = 2); Madeira River (Aripuana River (n = 2); Manicoré (n = 5); Humaitá (n = 7)); Roosevelt River (n = 1); Tapajos River (n = 1)	H9, H10, H11, H28, H43, H57, H60, H61, H62, H63, H64, H77, H82	No cases found

1% per million years was employed (Matzen da Silva et al., 2011; Olson et al., 2009). This represents an average of 1 mutation each 152,000 years. Thus, we have used an average of one mutation each 171,600 years for both *mt COI* and *COII*.

2.3.3. Demographic changes

We used several procedures to determine the possible historical population changes of the different *Lagothrix* taxa we analyzed. For example, we followed the method developed by Rogers and Harpending (1992) and Rogers et al. (1996) to create the mismatch distribution (pairwise sequence differences). We used the raggedness *rg* statistic (Harpending et al., 1993; Harpending, 1994) to determine the similarity between the observed and the theoretical curves. We used the Fu and Li D and F tests (Fu and Li, 1993), the Fu FS statistic (Fu, 1997), the Tajima D test (Tajima, 1989) and the R2 statistic of Ramos-Onsins and Rozas (2002) to determine possible population size changes in the *Lagothrix* sets we analyzed (Simonsen et al., 1995; Ramos-Onsins and Rozas, 2002). Also, a Bayesian skyline plot (BSP) was obtained using the BEAST v. 1.6.2 and Tracer v1.5 software for the taxa *lugens*, *lagotricha*, *poepigii* and *cana*. The Coalescent-Bayesian skyline option in the tree priors was selected with five steps and a piecewise-constant skyline model with 40,000,000 generations (the first 4,000,000 were discarded as burn-in). The marginal densities of temporal splits were analyzed in Tracer v1.5, and the Bayesian Skyline reconstruction option was selected for the trees log file. A stepwise (constant) Bayesian skyline variant was selected with the maximum time as the upper 95% HPD (High Posterior Density).

3. Results

3.1. Characteristics and evolutionary models of the sequences studied

The nucleotide frequencies were A = 31.84%, T = 28.78%, C = 26.37%, and G = 13.01% for the 141 sequences and 1,337 bp analyzed at the *mt COI* and *COII* genes. For the AIC procedure, the best evolutionary model was GTR (+G) with 10,528.296. The maximum likelihood estimate of transition/transversion bias was 3.41 for the GTR (+G) model (−4728.254). The estimated value of the shape parameter for the discrete Gamma Distribution was 1.034 for the GRT (+G) model (−4948.367).

3.2. Gene diversity and genetic heterogeneity within the genus *Lagothrix*

The main gene diversity statistics obtained for the *Lagothrix* genera as a whole were $NH = 82$, $H_d = 0.971 \pm 0.007$ and $\pi = 0.028 \pm 0.0035$ (Table 5). These values are important to consider if, for example, there is only one genus of woolly monkeys, *Lagothrix*, or two genera, *Lagothrix* and *Oreonax*.

Of the five taxa analyzed *poepigii* showed the highest levels of gene diversity ($NH = 40$, $H_d = 0.988 \pm 0.009$ and $\pi = 0.0266 \pm 0.0047$). Also, *lugens* yielded elevated levels of gene diversity ($NH = 15$, $H_d = 0.905 \pm 0.041$ and $\pi = 0.0409 \pm 0.0143$), although these genetic diversity levels clearly diminished when three individuals (presenting the most divergent sequences within this taxa) were extracted from the analysis ($NH = 12$, $H_d = 0.877 \pm 0.049$ and $\pi = 0.0301 \pm 0.0031$). The *cana* and *lagotricha* taxa showed similar gene diversity levels, considerably lower than those of *poepigii* and *lugens*. However, *L. flavicauda* showed the lowest levels of gene diversity and not show genetic variability at the *mtCOI*–*COII* genes.

All of the genetic heterogeneity statistics we used showed significant genetic differences among the five taxa of woolly monkeys studied (Table 6) taken as a whole and by taxa pairs (Table 7). However, the Kimura 2P genetic distances by taxa pairs were relatively small inside the Humboldt woolly monkey (Table 8). *Poepigii* was the taxon which showed the highest genetic distances with reference to the other taxa, being the highest genetic distance pair between *poepigii* and *cana* ($D = 0.0287$). The taxa pair with the lowest genetic distance was *lugens* and *lagotricha* ($D = 0.0165$). The average genetic distance among these four *Lagothrix* taxa was 0.0232 ± 0.0046 . *Flavicauda* revealed a higher average genetic distance with regard to the other taxa studied. The average genetic distance with reference to these other four taxa was 0.0326 ± 0.0041 , being the most differentiated taxon, with reference to *flavicauda*, *poepigii* ($D = 0.0385$) and the lowest differentiated taxon was *cana* ($D = 0.0291$). Thus, it is interesting to note that the nearest taxon geographically to *flavicauda*, *poepigii*, is also the most divergent from a genetics point of view.

3.3. Molecular phylogenetics of the genus *Lagothrix*

Five phylogenetic procedures were employed, but all of them showed similar results (Fig. 2). Herein we discuss the maximum likelihood (ML) (Fig. 1a) and the Bayesian (BY) (Fig. 1b) trees. Clearly, *flavicauda* was the most differentiated clade within *Lagothrix*. Both trees showed robust statistics with regard to this: a bootstrap of 99% for the ML tree and a posterior probability of $p = 1$ for the BY tree. After the separation of the ancestors of *flavicauda*, the ancestors of *cana* diverged (33% bootstrap and $p = 1$). Only animals with the phenotype and typical geographical origins reported for this taxon were observed within this *cana* clade. The ML tree only showed two *cana* groups with low-medium bootstrap percentages: one group with 57%, including exemplars from Tefé, Madeira, Roosevelt and Tapajos Rivers and one exemplar from the Acre state, and another group with 55%, including individuals from the Tefé and Purus Rivers. The BY tree yielded two separated clades for *cana* with $p = 1$, one including a specimen from the Tefé River and four individuals from the Madeira, Roosevelt and Tapajos rivers. Other minor clades with high posterior

probabilities included one comprised of four animals from the areas of Humaitá and Manicoré at the central Madeira River ($p = 0.98$), another integrated by four other animals from the same geographical area as in the previous case (Humaitá and Novo Aripuaná) ($p = 0.99$) and a third group integrated by five individuals from the Purus and Tefé Rivers ($p = 0.97$). Some of these smaller clades could have geographical significance. The ancestors of *poepigii* diverged next. Both trees showed three exemplars of *lugens* (one from the Caqueta Department and two from the Southern Antioquia Department in Colombia) within the *poepigii* clade. As these exemplars did not proceed from contact zones between *poepigii* and *lugens*, they probably represented cases of genetic introgression in the past and therefore are not recent cases of hybridization. The ML tree distinguished three clades with medium–high bootstrap percentages. One clade (89%) was composed of individuals from the Ucayali River (and tributaries as the Tapiche River) and from the Huallaga River (a tributary of the Marañón River) both in the Peruvian Amazon. The second group (51%, but internally many of the clusters yielded bootstraps higher than 85%) was also from the Peruvian Amazon and it geographically overlapped with the previous one. It contained individuals sampled near the Ucayali and Nanay rivers and the San Martín Department. A third little group (87%) was composed of two animals from the Yavari River at the Brazilian bank (frontier between Brazil and Peru). The BY tree also detected these three groups ($p = 0.84$, 0.7 and 1, respectively), but it also detected a fourth significant group ($p = 0.85$) conformed by animals from the areas of Napo and Pastaza Provinces of the Ecuadorian Amazon.

The last divergence split involved the ancestors of *lugens* and *lagotricha* ($p = 0.97$). Within *lugens* there appeared three possible cases of genetic introgression, with two individuals of *poepigii* (from the Napo and Ucayali rivers, Peruvian Amazon) and one *lagotricha* (from the Miriti-Paraná River at the Colombian Amazon). Also, there is a probably case of recent hybridization in one individual from the Guainia Department in Colombia. This exemplar showed an intermixed phenotype between *lagotricha* and *lugens* and it was sampled in a geographical area typically from *lagotricha* but its mitochondrial DNA was typical of *lugens*. In the ML tree, only one cluster showed an elevated bootstrap within *lugens* (79%). This group consisted of six individuals (two *poepigii* individuals within this clade, two from the Departments of Tolima and Huila, one from the Cundinamarca Department, and one from Southern Antioquia). These two last individuals and one from the Tolima-Huila Departments constituted a very divergent lineage within this group (93%). These three individuals were not enclosed in the BY tree and instead are being investigated to determine if they could constitute another *Lagothrix* taxon or if they corresponded to *lugens* individuals from isolated populations within the Colombian Andes mountains, which have experienced intense gene drift. The BY tree also detected this unique group with a very high posterior probability ($p = 1$), in this case conformed by the two *poepigii* and one individual from the Tolima-Huila Departments. The different clusters found within *lugens* did not show any geographical relationship among the individuals analyzed. Within *lagotricha*, two cases of genetic introgression and two cases of recent hybridization were detected. Two *lugens* individuals (from Huila-Tolima and Southern Antioquia Departments) were enclosed in this *lagotricha* clade (genetic introgression). However, there were two individuals from the Yasuni National Park in the Ecuadorian Amazon (a *poepigii* enclave) having a phenotype with a greater resemblance to *poepigii* but also having several traits of *lagotricha*. Additionally, it had *lagotricha* mitochondrial DNA (recent hybridization). Within the *lagotricha* clade, the ML tree only detected a very limited number of clusters with high bootstrap percentages: a group of two individuals from the Colombian

Amazon (Araucara-Caqueta Department and Amacayacu-Amazon Department; 95%), a group of two animals from the western bank of the Negro River in Brazil (63%) and another cluster of two individuals from the Vaupes and Putumayo Departments (67%). The BY tree also detected these three small groups ($p = 0.99$, 0.67 and 1, respectively) plus another little group conformed by three individuals from the Amazon River in Colombia (El porvenir), the western bank of the Negro River and one of the mixed individuals from the Yasuni National Park in Ecuador ($p = 0.76$). Similar to one of the previously discussed clades, we did not detect any relationship between genetic and geographical distances within *lagotricha*.

These results indicate *poepigii* as the *Lagothrix* taxa within more significant inner groups, and that these groups are related to different geographical areas.

3.4. Temporal divergence splits among *Lagothrix* taxa

The temporal divergence splits obtained with the ρ statistic and the MJN procedure were as follows: The largest temporal split between *flavicauda* and the other taxa was with *lagotricha* ($2,971,000 \pm 148,570$ YA) and the lowest one with *lugens* ($2,021,000 \pm 106,360$ YA). On average, the split between *flavicauda* and all the other *Lagothrix* taxa was around $2,413,700 \pm 160,740$ YA. The temporal splits for the taxa traditionally classified within *Lagothrix lagotricha* were lower than those between *flavicauda* and the other *Lagothrix* taxa. For instance, the temporal splits between *lagotricha* and *poepigii*, *lagotricha* and *cana* and *lagotricha* and *lugens* were $1,418,180 \pm 141,820$ YA, $1,075,860 \pm 107,590$ YA and $992,730 \pm 141,820$ YA, respectively. The diversification of the mitochondrial haplotypes within *poepigii* began around $1,677,000 \pm 152,770$ YA. Haplotype diversification first began at this taxon. *Lucens* (the three most differentiated individuals were deleted in this analysis) showed a haplotype diversification around $780,000 \pm 138,840$ YA, whereas the two taxa with the most recent haplotype diversification were *cana* and *lagotricha* with $474,500 \pm 67,550$ YA and $320,500 \pm 49,130$ YA, respectively.

3.5. Demographic evolution in *Lagothrix*

The demographic evolution of the different *Lagothrix* taxa are shown in Table 9 and Figs. 2 and 3. The mismatch distribution analyses showed that only the *poepigii* taxa clearly yielded a significant population expansion. The genus as a whole, *lugens*, *cana* and *lagotricha* did not show significant evidence of population expansion with this analysis. However, different procedures revealed stronger evidence in favor of population expansion in these *Lagothrix* taxa. The *Lagothrix* genus taken as a whole showed five statistics with significant values agreeing with a population expansion (Tajima D, Fu and Li F^* and D^* , Fu F_s and R2 statistics). *Poepigii* and *lagotricha* also showed these five statistics significantly in agreement with a population expansion. Only three tests (Tajima D, Fu and Li F^* and D^*) indicated significant population expansion for *lugens* and *cana*. Fu F_s and R2 are the more powerful statistics in detecting real population expansions (Ramos-Onsins and Rozas 2002). Only the *Lagothrix* genus as a whole, and *poepigii* and *lagotricha* showed clear signatures of population expansion in their mitochondrial genes. The monomorphism found in *L. flavicauda* revealed that this taxon has experienced a very extreme population bottleneck, different than the other *Lagothrix* taxa (see Fig. 4).

The BSP analysis revealed the following trends of the four *Lagothrix* taxa analyzed. For *poepigii*, there has been a constant gradual population expansion over the last one MY, although this population expansion has intensified in the last 0.75 MY. *Cana* showed a constant female population effective size during the last 700,000 years with a slight population increase in the last

Table 5

Different gene diversity statistics in *Lagothrix flavicauda*, in *Lagothrix lagotricha* and in four subspecies of this last species at the mt *COI* and *COII* gene sequences. NS = Number of sequences; NSS = Number of segregating sites; NH = Number of haplotypes; H_d = Haplotype diversity and standard deviation; K = Average number of differences; π = Nucleotide diversity and standard deviation and the statistic θ per sequence.

Group	NS	NSS	NH	H_d	K	π	θ
<i>Lagothrix flavicauda</i>	8	0	0	0.000 ± 0.000	0	0.0000 ± 0.0000	0
<i>Lagothrix lagotricha</i>	133	376	82	0.971 ± 0.007	19.714	0.0280 ± 0.0035	68.353
<i>L. l. lugens</i>	26	230	15	0.905 ± 0.041	29.185	0.0409 ± 0.0143	60.273
<i>L. l. lagotricha</i>	30	53	16	0.789 ± 0.080	4.74	0.0067 ± 0.0021	13.378
<i>L. l. poeppigii</i>	47	233	40	0.988 ± 0.009	19.973	0.0266 ± 0.0047	52.754
<i>L. l. cana</i>	30	61	14	0.823 ± 0.063	5.52	0.0077 ± 0.0026	15.398

Table 6

Diverse genetic heterogeneity statistics (χ^2 , H_{ST} , K_{ST} , K_{ST}^* , Z_S , Z_S^* , S_{nn}) and their associated probabilities for the different *Lagothrix* taxa (*flavicauda*, *lugens*, *lagotricha*, *poeppigii*, *cana*) studied at the mt *COI* and *COII* genes. The three most divergent *lugens* sequences were extracted of this analysis. All the probability values were significant ($^*p < 0.00001$). Also some gene flow estimates (N_m) and the genetic heterogeneity statistics from which derived are shown.

Genetic differentiation estimated	p	Gene flow	
$\chi^2 = 512.274$ $df = 312$	0.00001*	$G_{ST} = 0.1319$	$N_m = 3.29$
$H_{ST} = 0.1155$	0.00001*	$\gamma_{ST} = 0.3936$	$N_m = 0.77$
$K_{ST} = 0.3812$	0.00001*	$N_{ST} = 0.6087$	$N_m = 0.32$
$K_{ST}^* = 0.2934$	0.00001*	$F_{ST} = 0.6086$	$N_m = 0.32$
$Z_S = 2714.207$	0.00001*		
$Z_S^* = 7.154$	0.00001*		
$S_{nn} = 0.8687$	0.00001*		

Table 7

Genetic heterogeneity statistics among *Lagothrix* taxa pairs at the mt *COI* and *COII* genes. G_{ST} above of the main diagonal and F_{ST} below of the main diagonal.

Taxa	<i>L. flavicauda</i>	<i>L. l. lagotricha</i>	<i>L. l. lugens</i>	<i>L. l. poeppigii</i>	<i>L. l. cana</i>
<i>L. flavicauda</i>	–	0.199	0.202	0.119	0.197
<i>L. l. lagotricha</i>	0.893	–	0.080	0.051	0.116
<i>L. l. lugens</i>	0.794	0.401	–	0.028	0.088
<i>L. l. poeppigii</i>	0.662	0.372	0.266	–	0.054
<i>L. l. cana</i>	0.881	0.662	0.528	0.426	–

Table 8

Kimura 2-P genetic distance among different *Lagothrix* taxa pairs at the mt *COI* and *COII* genes (in percentages %).

Taxa	<i>L. flavicauda</i>	<i>L. l. lagotricha</i>	<i>L. l. lugens</i>	<i>L. l. poeppigii</i>	<i>L. l. cana</i>
<i>L. flavicauda</i>	–				
<i>L. l. lagotricha</i>	3.05%	–			
<i>L. l. lugens</i>	3.19%	1.65%	–		
<i>L. l. poeppigii</i>	3.85%	2.59%	2.67%	–	
<i>L. l. cana</i>	2.91%	1.99%	2.13%	2.87%	–

100,000 years. *Lagothrix* experienced a strong population expansion in the last 300,000 YA until today, while *lugens* showed a constant female effective population size until 25,000 YA, when a clear population decrease was observed. This female population decrease in *lugens* was also detected by the $F_u F_s$ statistic. Therefore, there is complete agreement between the neutrality tests for determining possible population changes and the BSP analyses. The two taxa with clear and strong population expansions were *poeppigii* and *lagotricha*.

4. Discussion

4.1. How many woolly monkey species and genera are there?

The answer of this question can vary depending on the species criteria used. For example, the biological species concept (BSC), is

arguably the strongest concept of species for sexually reproducing taxa (Mayr, 1942, 1963). This concept holds that species are sets of interbreeding populations that are reproductively isolated (with no fertile hybrids) from other similar sets. This means that this concept has taken into account the existence of pre-zygote isolation reproductive processes, which is a paradigm within the Neodarwinism synthesis (Barton and Bengtsson, 1986; Coyne et al., 1994; Antonovics, 2006). The BSC has two major operational problems. First, it is difficult to evaluate among populations in extreme allopatry. Second, the researchers must demonstrate if there are fertile hybrids among different taxa in the wild. Other alternative species definitions are the genetic species concept (GSC; Baker and Bradley, 2006) and the phylogenetic species concept (PSC; Cracraft, 1983). The first one defines a species as a group of populations that are genetically isolated from other groups (two different genetic pools with independent evolutionary fates). Thus, this definition does not necessarily imply pre-zygote isolation reproductive processes. The second one defines a species as the smallest monophyletic and diagnosable cluster of individuals with a parental pattern of ancestry and descent by means of molecular or morphological characteristics. Operationally, this concept is easier to apply than the BSC.

Many authors are applying this concept in many different organismal taxa, including mammals. Nevertheless, the large-scale application of this concept in mammals has originated many “crazy” new species that are clearly not sustained by a critical analysis. This was named by Isaac et al. (2004) as “taxonomic inflation.” Zachos et al. (2013) suggests that some of the proposed new mammal species are completely unjustifiable (new species of tigers as *Panthera sumatrae* and *P. sondaica*, Cracraft et al., 1998; Mazak and Groves, 2006; 12 new species of red deer, *Cervus elaphus*, Groves and Grubb, 2011; 11 new species of the small rocky antelope, *Oreotragus oreotragus* or new species of hares, *Lepus*, Palacios et al., 2008).

Unfortunately, this “revolution” has arrived and affected the world of primatology. Ever since the publication of Groves's (2001) book, PSC is now being widely embraced by primatologists. For instance, Napier (1976) recognized 67 platyrrhine species, while Groves (2001) recognized 112 platyrrhine species. Rylands et al. (1993) recognized five genera and 27 species of Callitrichine (callimico, tamarins and marmosets), whereas Rylands and Mittermeier (2009) recognized seven genera and 42 species for the same Neotropical primate group. An additional example could be the case of *Cebus albifrons* (white-fronted capuchin). While this taxon has traditionally been considered one species with several subspecies (Hernández-Camacho and Cooper, 1976; Ruiz-García et al., 2010), applying PSC, Boubli et al. (2012) have defined at least nine different species of white-fronted capuchins. There is, however, clear evidence of interbreeding among different *C. albifrons* taxa in captivity and also in the wild. Interbreeding has also occurred in the same troop within the wild evidenced by several different taxa morphotypes being seen together (Hernández-Camacho and Cooper, 1976; Ruiz-García, personal observation). The case of *Callicebus* is also interesting from this point of view.

Hershkovitz (1963) recognized two species of *Callicebus* with a total of 13 taxa. The same author in 1990 (Hershkovitz, 1990) recognized within *Callicebus*, 13 species and 25 taxa. Many of them were probably new taxa due to our poor knowledge of this genus. More recently, van Roosmalen et al. (2002) used a typical typology viewpoint of taxonomy and ended up considering 28 full species of *Callicebus*. Such is the current situation of modern primatology.

To determine how many genera and species of woolly monkeys there are, we start with the following principle: The general consensus among scientists regarding humans is that there is only one species (*Homo sapiens*) because interbreeding has been clearly demonstrated. This does not imply that morphological and molecular differences do not exist among different human groups (using monophyly in phylogenetic analyses). They do exist. Given this fact, even different human families in different apartments in a block within a determined city could be different species. Surely they have some morphological and molecular traits not possessed by other families on the same block! Similarly, it would be inaccurate and even foolish to apply this viewpoint to dogs because each dog race has unique morphological or molecular attributes. However, there is no attempt to apply the PSC indiscriminately in humans or in domestic animals which we perfectly know, as has been done to many Primates taxa in the last few years. If this is the view for our own primate species, then we must disagree with other scientists that apply different species rules for other primate species. Additionally, an indiscriminate application of PSC could generate an unmanageably large number of species. We would be negligent to forget the great number of problems and confusion caused by using primitive typological classifications in the past (see as an example, the case of Merriam, 1918, with 86 brown bears species supposedly in North America). The primatologists, who follow the PSC, have upgraded all the primate subspecies to species, but we consider that it is only moving the problem up one level as it obscures the reality of a real evolutionary unit.

The Kimura 2P genetic distances we obtained among *flavicauda* and the other *Lagothrix* taxa do not support the taxonomic conclusions presented by Groves' (2001). The average genetic distance obtained between *flavicauda* and the other four *Lagothrix* taxa was $3.26\% \pm 0.41\%$ (maximum value 3.85%). For *mtCOII*, Ascunce et al. (2003) showed the following genetic distances among different South American primate genera: *Alouatta*–*Brachyteles* (16%), *Ateles*–*Brachyteles* (12%), *Lagothrix*–*Brachyteles* (15.2%), *Alouatta*–*Ateles* (14.9%), *Cebus*–*Saimiri* (16%), and *Cebus*–*Aotus* (17%). For *mtCOI*, Lim (2012) determined an average genetic distance among different genera of Didelphimorphia (9.3%) and Echimyidae (Rodentia) (12.82%). Agrizzi et al. (2012) showed an average genetic distance of 11.2% at *mtCOI* (10.8% at *mtCyt-b*) among species within a genus within Didelphidae (Marsupialia). Patton et al. (2000) also showed species within the genera *Marmosa* and *Micoureus* with average genetic distances of 15%. The average genetic distances at the *mtCOI* gene among some genera of Artiodactyla, such as *Oryx*–*Capra* or *Oryx*–*Cephalophus*, were 12.6% and 13.7%, respectively (Elmeer et al., 2012). Kartavtsev (2011) analyzed sequences of *mtCOI* from 20,731 vertebrate and invertebrate animal species. He estimated the average distance data for five different groups. He obtained $0.89\% \pm 0.16\%$ for populations within species, $3.78\% \pm 1.18\%$ for subspecies or semi-species, $11.06\% \pm 0.53\%$ for species within a genus; $16.60\% \pm 0.69\%$ for species from different genera within a family and $20.57\% \pm 0.40\%$ for species from separate families within an order.

Interestingly, Bradley and Baker (2001), focused their work on *mtCyt-b*, a gene with a similar behavior to the two mitochondrial genes we studied. They claimed values $<2\%$ would equal intra-specific variation, values between 2% and 11% would merit additional study, and values $>11\%$ would be indicative of specific recognition.

The estimated average genetic distance between *flavicauda* and other *Lagothrix* taxa is around 4–6 times lower than values found among different genera within different orders of Mammals for both mitochondrial genes (*COI* and *COII*). Thus, in our criteria, *flavicauda* is one species within *Lagothrix* (*Lagothrix flavicauda* sensu Fooden, 1963) although its genetic divergence is within the typical subspecies range with regard to other *Lagothrix* taxa, (Kartavtsev, 2011).

Despite this, we consider *flavicauda* as a full species. We are simultaneously applying the BSC, GSC, and PSC. For the BSC, it is an allopatric situation with reference to the other *Lagothrix* taxa. There are no known hybrids in the wild nor in captivity with these other *Lagothrix* taxa. In regard to the GSC, no mitochondrial haplotype is shared with the other *Lagothrix* taxa. Finally, for the PSC, mitochondrial sequences make up a monophyletic group and it has several physical characteristics which are not shared with other *Lagothrix* taxa: (1) General color of deep mahogany; (2) Face with a clearly defined buffy nasal patch; (3) Very intense yellow hairs on scrotum and distal half of under tail surface; (4) Squamosal process of zygomatic twice as deep as temporal; (5) Braincase narrow but zygomatic arches wide; (6) Medial superciliary protuberances prominent. Nevertheless, the genera status is not confirmed by our molecular population analysis, thus disagreeing with Groves (2001) and agreeing with Mathews and Rosenberger (2008a,b). This means that the morphological differences between *L. lagotricha* and *L. flavicauda* proposed by Groves (2001) to separate both *Lagothrix* species in two different genera have no phylogenetic value, at least, at the genus level. The inclusion of characters experiencing environmental natural selection and morphological plasticity is a detriment to good phylogenetic resolution. We agree with the comments of Mathews and Rosenberger (2008a,b) on this topic.

Our results, in disagreement with Groves (2001), suggest that *L. lugens*, *L. lagotricha*, *L. poeppigii* and *L. cana* are not full species. We have three argument lines to support this disagreement. First, the average genetic distances among these four *Lagothrix* taxa was $2.32\% \pm 0.46\%$, with the highest value between *poeppigii* and *cana* (2.87%). Clearly, these values were significantly lower than the values obtained for other well recognized species of Neotropical primates. For instance, Ascunce et al. (2003) reported, at the *mtCOII* gene, an average genetic distance of 5.76% among *Alouatta* species and 7.4% among *Aotus* species. Collins and Dubach (2000b) reported an average genetic distance of 4.3% (with a maximum of 4.7%) among *Ateles* species, also at the *mtCOII* gene. At the *mtCOI* gene, Agrizzi et al. (2012) estimated an average intraspecific genetic divergence of around 2.0% (1.9% at the *mtCyt-b*) for species of Didelphidae. Some individuals even showed high levels of genetic distances for *mtCOI* within their respective species as in the cases of *Micoureus demerarae* (5%; range: 0–8.4%), *Gracilianus microtarsus* (4.2%; range: 0.5–7.2%) and *Metachirus nudicaudatus* (3.9%; range: 0–9.2%). In Artiodactyla, for instance, Elmeer et al. (2012) estimated an average genetic distance at the *mtCOI* of 4.8% among three species of *Oryx* (*O. dammah*, *O. leucoryx*, *O. gazella*). The reported behavior of both mitochondrial genes considered here is very similar, although for some species (humans and dogs) *COII* showed more gene diversity than *COI*, but for other species (cow and pig) *COI* showed more diversity than *COII* (Santamaria et al., 2007). Thus, the average genetic distance among the four *Lagothrix* taxa are 2–3 times lower than these estimates for full species in different groups of mammals for both mitochondrial genes studied. Collins and Dubach (2000b) estimated an average genetic distance of 2.69% (maximum of 3.07%) among subspecies of *Ateles*, being this value practically identical to what we found for these *Lagothrix* taxa as well as that determined by Kartavtsev (2011) for a large collection of different vertebrate and

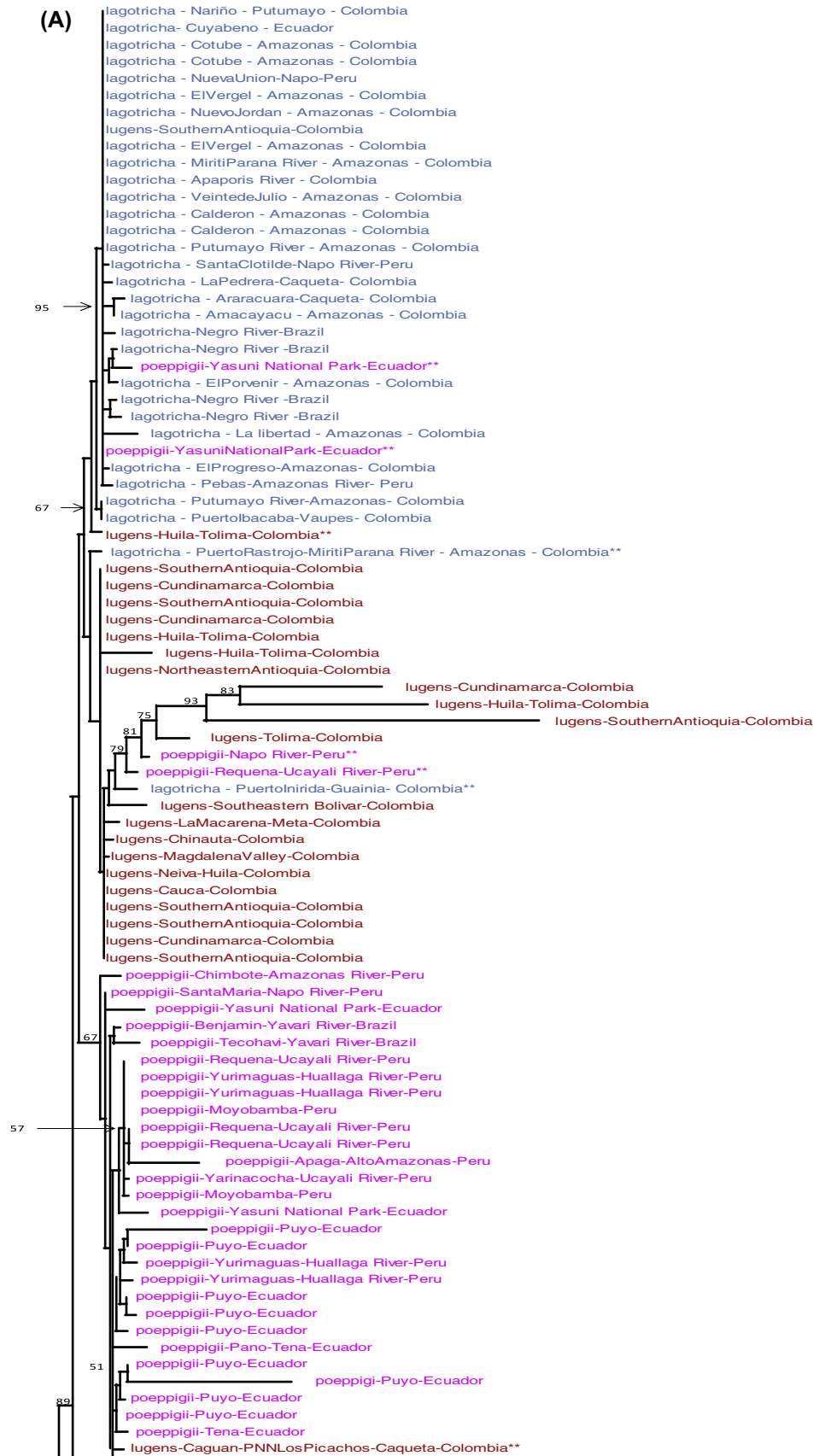


Fig. 1. (A) Maximum likelihood tree for the 141 *Lagothrix* analyzed at the *mt COI-COII* genes. Numbers in the nodes are bootstrap percentages (only values higher than 50% were shown). (B) Bayesian tree for 138 *Lagothrix* specimens (the three most differentiated *L. l. lugens* were deleted) sequenced at the *mtCOI-COII* genes. Numbers in the nodes are posterior probabilities (only values higher than 0.5 were shown). Individuals with asterisks are those which are probably hybrids or with genetic introgression from other taxa. Sequences of *Alouatta*, *Ateles* and *Brachyteles* were employed as out-groups. Blue = *L. l. lagotricha*; pink = *L. l. poeppigii*; red = *L. l. lugens*; green = *L. l. cana*; orange = *L. l. flavicauda*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Fig. 1 (continued)

invertebrate taxa ($3.78\% \pm 1.18\%$ for subspecies). Thus, the genetic distances we found agree quite well with four subspecies considered by Fooden (1963).

Secondly, our molecular phylogenetic trees and the morphological traits of the animals studied clearly revealed the existence of recent hybrids and historical genetic introgression, at least, among three of these *Lagothrix* taxa. We detected eight cases of genetic introgression and three cases of recent hybridization. Three *lugens* appeared in the *poeppigii* clade. These animals showed typical *lugens* phenotypes, but with *poeppigii* mitochondrial DNA, and they were sampled in areas far away from *poeppigii*'s current range. Most likely, these three cases represent mitochondrial DNA introgression from *poeppigii* into the *lugens* lineage because the haplo-

type diversification in *poeppigii* seems to be older than the mitochondrial haplotype diversification in *lugens*. Two additional cases of historical genetic introgression were the presence of two *poeppigii* individuals within the *lugens* clade. These two individuals showed a typical *poeppigii* phenotype and they were sampled far away from *lugens*'s geographical range. This could be interpreted as a case of mitochondrial genetic introgression from *lugens* within *poeppigii*. However, as the diversification of *poeppigii* is older than that of *lugens*, it could also be interpreted that these two *poeppigii* individuals have ancestral haplotypes that contributed to the origin of the current *lugens* haplotypes. Another case of genetic introgression was observed by the presence of a *lagotricha* individual (with a typical phenotype of this taxon) within the *lugens* clade

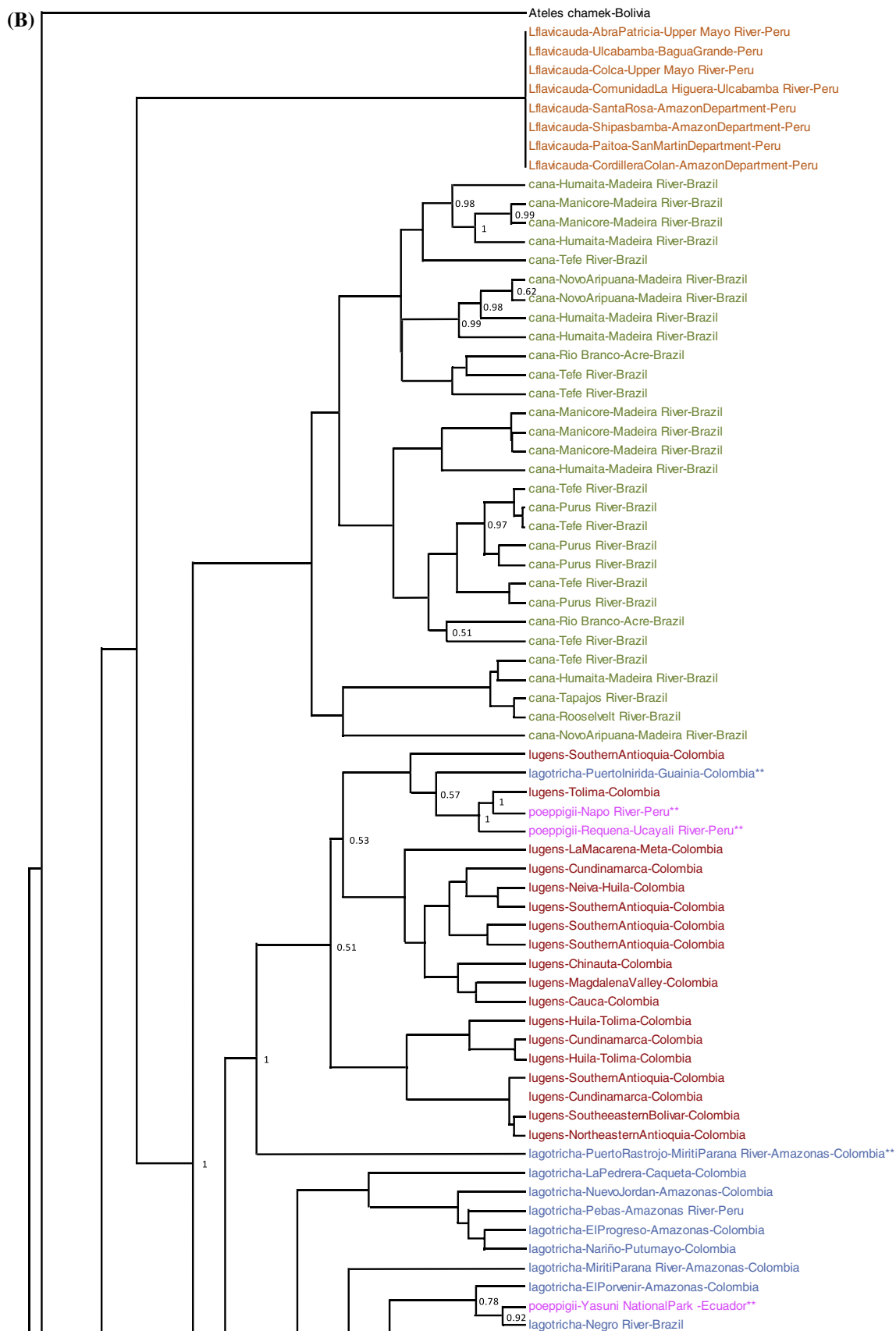


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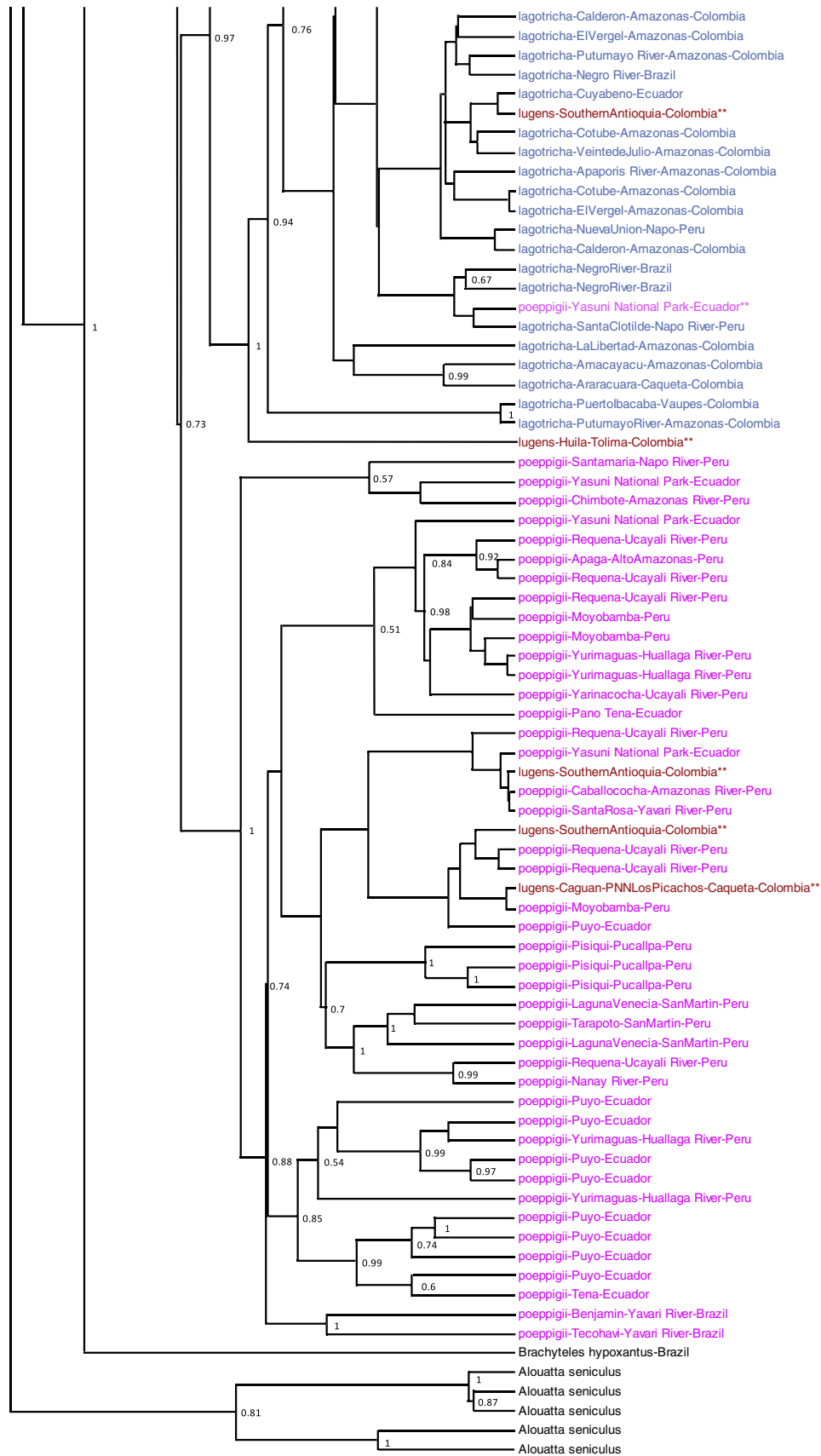


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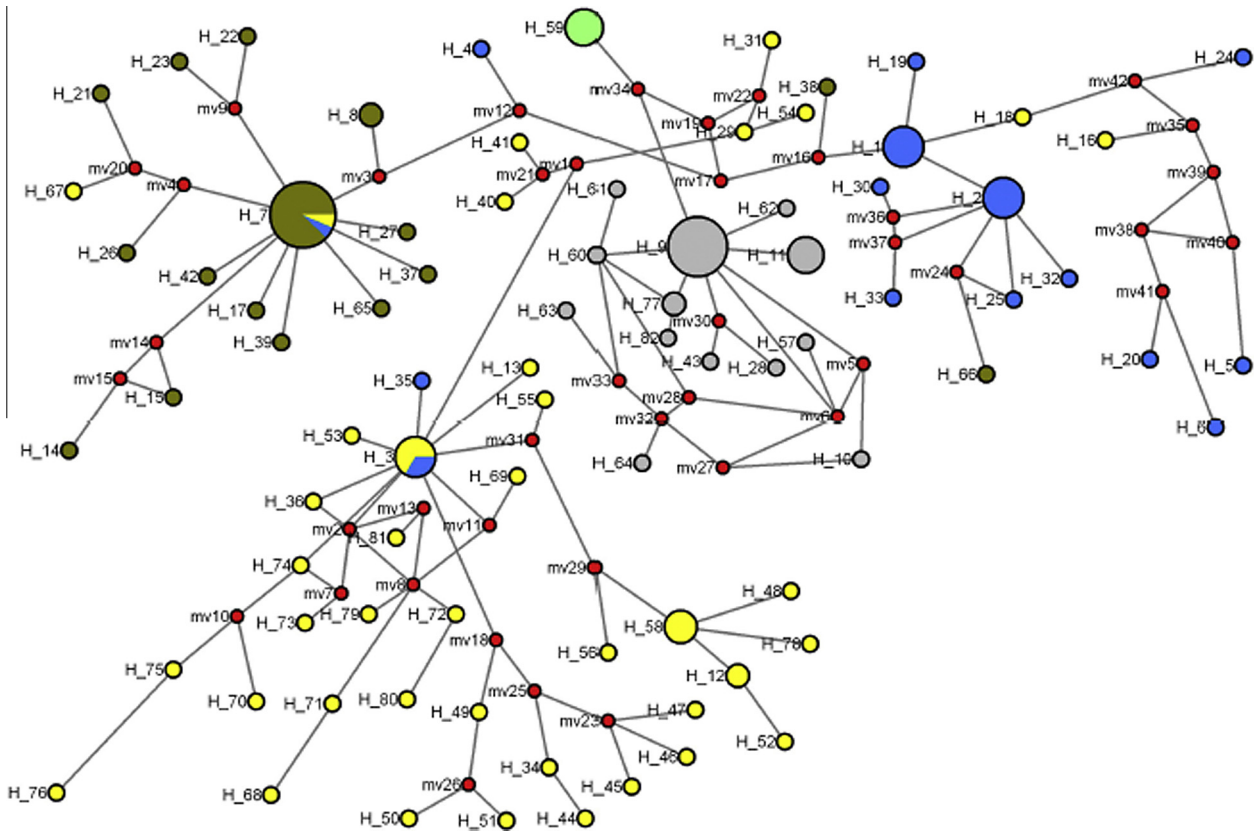


Fig. 2. Median Joining Network with the 82 mitochondrial haplotypes found in this study for the *Lagothrix* genus analyzed at the *mt COI-COII* genes. In red, possible haplotypes which were not sampled or that they were disappeared. Blue = haplotypes of *L. l. lugens*; brown = haplotypes of *L. l. lagotricha*; yellow = haplotypes of *L. l. poeppigii*; grey = haplotypes of *L. l. cana*; Green = Unique haplotype of *L. flavicauda*. Some haplotypes of *lugens*, *lagotricha* and *poeppigii* are intermixed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 9

Demographic statistics applied to the *Lagothrix* genus and to the *L. lagotricha* subspecies studied. * $p < 0.05$; ** $p < 0.001$, significant population expansions.

Taxa	Tajima D	Fu and Li D*	Fu and Li F*	Fu's Fs	Raggedness rg	R2
<i>Lagothrix</i> genus	P[D ≤ -2.559] = 0.000**	P[D* ≤ -6.322] = 0.000**	P[F* ≤ -5.455] = 0.001**	P[Fs ≤ -32.580] = 0.000**	P[rg ≤ 0.0064] = 0.263	P[R2 ≤ 0.0273] = 0.000**
<i>L. l. lugens</i>	P[D ≤ -2.249] = 0.001**	P[D* ≤ -2.943] = 0.015*	P[F* ≤ -3.205] = 0.004*	P[Fs ≤ 3.628] = 0.893	P[rg ≤ 0.0250] = 0.830	P[R2 ≤ 0.0855] = 0.117
<i>L. l. lagotricha</i>	P[D ≤ -2.487] = 0.000**	P[D* ≤ -3.705] = 0.001**	P[F* ≤ -3.901] = 0.001**	P[Fs ≤ -4.673] = 0.004*	P[rg ≤ 0.0551] = 0.450	P[R2 ≤ 0.0601] = 0.006*
<i>L. l. cana</i>	P[D ≤ -2.479] = 0.000**	P[D* ≤ -3.933] = 0.001**	P[F* ≤ -4.079] = 0.000*	P[Fs ≤ -2.002] = 0.217	P[rg ≤ 0.023] = 0.115	P[R2 ≤ 0.077] = 0.072
<i>L. l. poeppigii</i>	P[D ≤ -2.479] = 0.000**	P[D* ≤ -4.603] = 0.001**	P[F* ≤ -4.566] = 0.001**	P[Fs ≤ -15.297] = 0.001**	P[rg ≤ 0.0031] = 0.001**	P[R2 ≤ 0.049] = 0.003*

in a geographical area far removed from the *lugens*'s range. This could be interpreted as a case of genetic introgression from *lugens* within *lagotricha* (which seems to be the most recent *Lagothrix* taxon with mitochondrial diversification). The presence of two *lugens* individuals with typical phenotypes of this taxon and a long way from *lagotricha*'s range could be due to historical genetic introgression from *lagotricha* within *lugens*. But there are also three cases of recent hybridization. For example, there was a phenotypically mixed individual (with more prominent characters of *lagotricha*) having *lugens* mitochondrial DNA within a *lagotricha* area. It was relatively near the range of *lugens*. Additionally, there were two individuals with mixed phenotypes from an area of contact between *lagotricha* and *poeppigii* in the Ecuadorian Amazon (but with more resemblance to *poeppigii*). They had *lagotricha* mitochondrial DNA.

The third compelling reason to consider *Lagothrix* taxa as subspecies was the relatively small divergence times found by the application of the ρ statistic on the haplotype network.

Having taken into account these three facts (small genetic distances, extensive genetic introgression and recent hybridization and relatively recent times of mitochondrial diversification), we consider that these four *Lagothrix* taxa are subspecies as claimed by Fooden (1963) (*L. l. lugens*, *L. l. lagotricha*, *L. l. poeppigii* and *L. l. cana*). They are not full species as suggested by Groves (2001), especially in this case, because the BSC is operational and no reproductive isolation was found in the wild. Henceforth, we consider woolly monkeys to only have one genus (*Lagothrix*) with two species (*lagotricha* and *flavicauda*) with the first one containing four subspecies. Another alternative possibility is to apply a mixed definition of BSC and PSC. If we do this, we can consider the *Lagothrix* genus with three species: *L. flavicauda*, *L. cana* and *L. lagotricha*. In this scenario, the last species would have three subspecies (*lugens*, *lagotricha* and *poeppigii*). This could be the result of the *cana* clade being monophyletic and that there was no genetic introgression or recent hybridization detected in the sample analyzed. However, the genetic distances with regard to the other *Lagothrix* taxa were

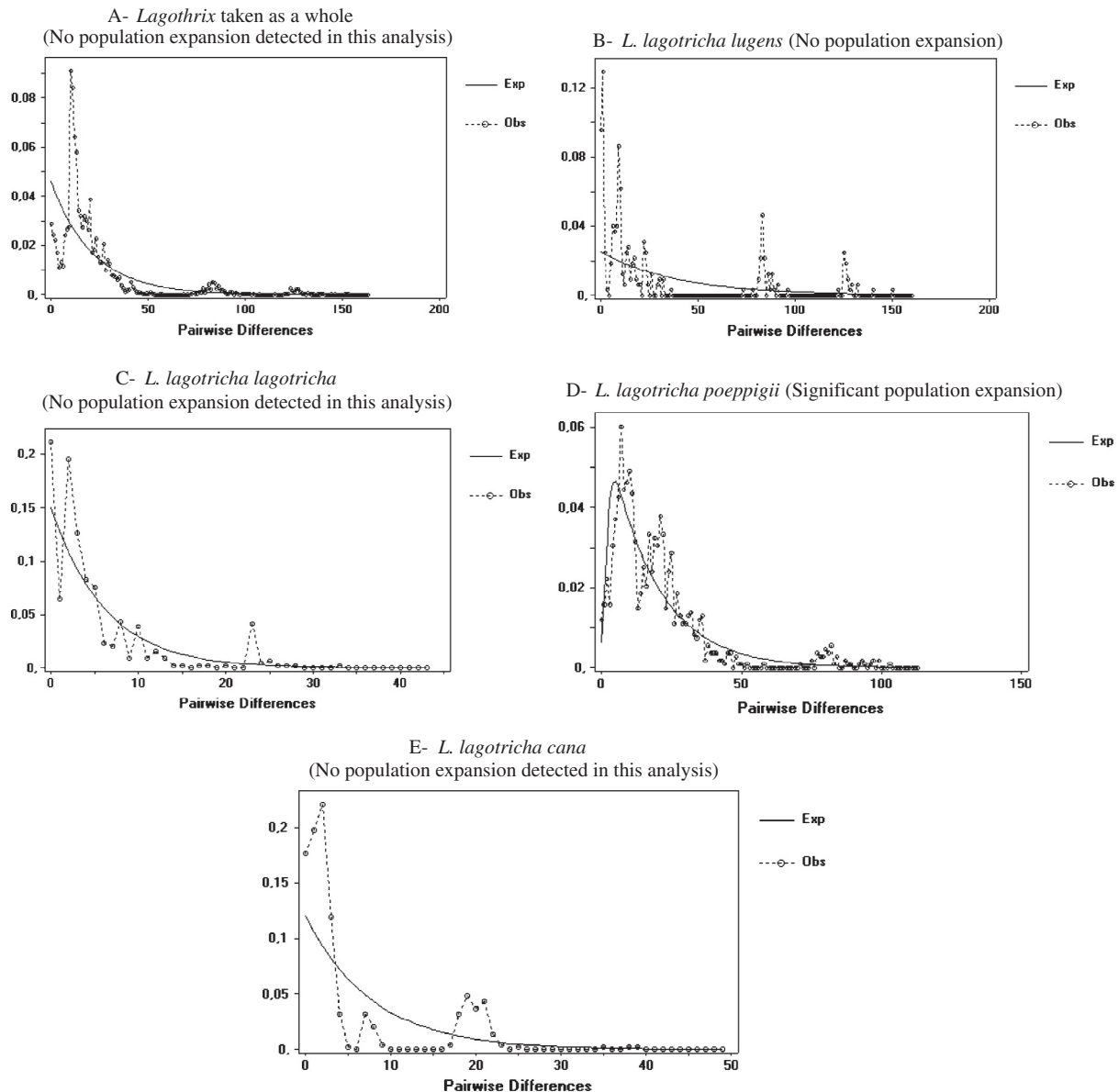


Fig. 3. Historical demographic analyses by means of the mismatch distribution procedure (pairwise sequence differences) at the mt *COI–COII* gene sequences studied in different *Lagothrix* taxa. These analyses were applied to: (A) *Lagothrix* taken as a whole; (B) *L. l. lugens*; (C) *L. l. lagotricha*; (D) *L. l. poeppigii*; (E) *L. l. cana*.

small. We have sampled *cana* individuals at the core of its geographical distribution. Perhaps, if we could sample in the peripheral distribution of this taxon (eastern bank of the Juruá River and along the left bank of the upper Ucayali River to the lower Pachitea River; see Peres, 1993 and Groves, 2001), some cases of genetic introgression and/or recent genetic hybridization, with *poeppigii*, could be detected. If this was the case, the BSC could clearly be operational and only two species would be recognized for *Lagothrix* (*flavicauda* and *lagotricha*).

Groves (2001) considered several morphological subspecies within what he had considered full species, *L. cana* and *L. poeppigii*. For example, he used the name *L. cana tschudii* for the animals from Northern Bolivia and Southern Peru (Cuzco). These exemplars were much darker than other *cana* individuals. They torsos were a deep blackish gray, in contrast to their practically black head, limbs and tail. The first author sampled one individual with these characters near Cuzco (2007). Also, a sample of one individual from Northern Bolivia was sent by Dr. R. Wallace to the first author.

Unfortunately, neither sample worked well. Samples such as these could be analyzed to determine the real status of this supposed subspecies. Groves (2001) also distinguished two supposed subspecies within *L. poeppigii*: *L. p. poeppigii* and *L. p. castelnaui* by noting small differences in coloration. We analyzed animals with these same color differences and they were intermixed in the phylogenetic analyses. Thus, we consider these minor color variations as not important from a phylogenetic point of view (individual variation within populations).

Recently, Mantilla-Meluk (2013) named two other possible subspecies of woolly monkeys within *L. lugens*. He followed the full species status for *lugens*—based on the work of Groves (2001), which our data do not support. After analyzing a modest sample size of individuals from the Field Museum of Natural History of Chicago for coat color and some skull morphometric variables, he concluded the existence of three subspecies within *lugens*. Fooden (1963) had already detected three morphotypes within *lugens* and these were exactly the same as what Mantilla-Meluk

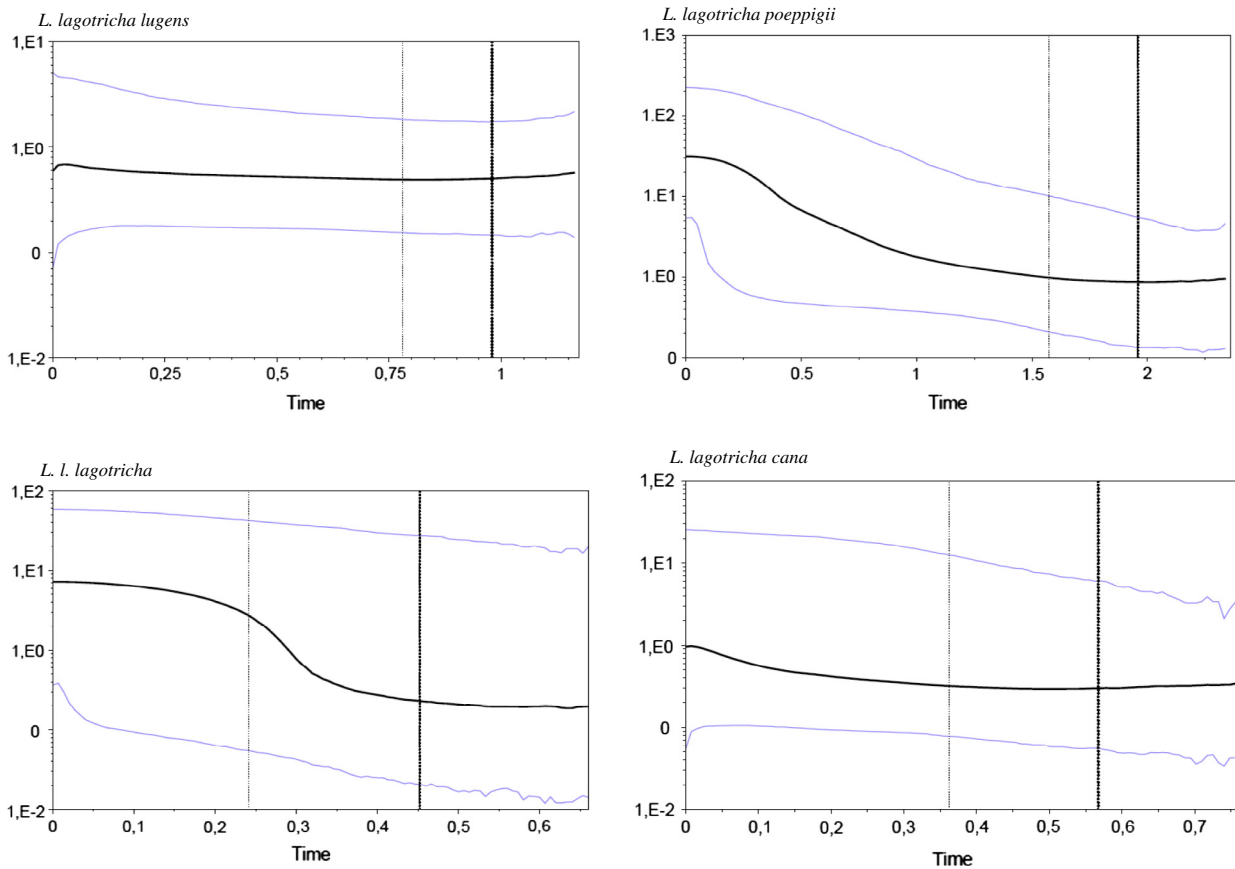


Fig. 4. Demographic Bayesian Skyline Plot analyses for the *Lagothrix* genus and for the taxa *lugens*, *lagotricha*, *poeppigii* and *cana*. Numbers on X axis are millions of years. Numbers on Y axis are female effective numbers.

had reported as new in 2013. There were *L. lugens lugens*, *L. lugens defleri* and *L. lugens sapiens*. We have four basic arguments against the Mantilla-Meluk point of view. First, we have sequenced both mitochondrial genes studied here from *lugens* individuals belonging to these three areas where supposedly, three *lugens* subspecies lived and no geographical clusters were obtained. We have even observed intermixed individuals of these three Colombian Andean regions in the same cluster. Second, although Mantilla-Meluk (2013) suggests to follow the GSC, he seems to be utilizing an extreme typological PSC. Unfortunately, the paper only considers coat color and some skull morphometric variables. Coat coloration is extremely affected by environmental natural selection. For instance, the very dark color and long hair of *lugens lugens* is also found in *tschudii* of Southern Peru and Bolivia because both populations live in mountainous high altitudes—enduring cold temperatures. Also, regrettably, the paper does not provide the genetics of these characters—causing the author to ignore if these three morphotypes really represent three different gene pools as needed by the GSC. Third, the paper ignores the possibility of hybridization among these three morphotypes. It probably occurs extensively. If it occurs between *lugens* and *lagotricha*, it occurs even more readily among different populations within *lugens*. Finally, fourth the paper does not show monophyly for each of the supposed taxa, either for morphological traits or for mitochondrial and nuclear genes. Although we applaud the concerted effort of primatologists studying morphometric traits, many times, these studies will benefit from the inclusion of genetic data. Thus, it is more logical to consider, as did Fooden (1963), the existence of three morphotypes within the subspecies *L. l. lugens* until a comprehensive study can be completed of these populations.

4.2. Gene diversity, evolution of each *Lagothrix* taxon, and conservation status of *L. flavicauda*

Our gene diversity and phylogenetic analyses revealed some closely related results. Although the ancestors of *cana* were the first to diverge within *L. lagotricha*, this fact was not revealed by the work of Ruiz-García and Pinedo (2010), the older diversification of current mitochondrial haplotypes was in *poeppigii* (1.6 MYA), which agrees quite well with the fact that *poeppigii* has the highest levels of gene diversity of all the *Lagothrix* taxa. This period of mitochondrial diversification in *poeppigii* is within the range of the beginning of the Pleistocene in different areas of the earth (2.5–1.6 MYA). This was the taxon which could have a greater number of significant lineages related to different geographical areas in the upper Amazon. We detected four geographical groups, two overlapping in the area of the Ucayali and Huallaga rivers and San Martín Department (Peru), another in the Peruvian area of the Yavari River (frontier with Brazil) and another in the Ecuadorian Amazon. This more noteworthy geographical structure is probably related to the older mitochondrial haplotype diversification of this *Lagothrix* taxon with regard to the other *Lagothrix* taxa. Although this taxon has elevated levels of mitochondrial gene diversity it was classified by IUCN in 2014 in the category of Vulnerable A2cd with a decreasing population trend.

The second taxon with higher levels of gene diversity was *lugens* (of the three extremely divergent sequences found for this taxon, none were related to the three different subspecies of *lugens* Mantilla-Meluk (2013) supposedly analyzed). Furthermore, its mitochondrial haplotype diversification began around 0.78 MYA. From 1.3 to 0.7 MYA, there was a very cold and dry glacial period

named Pre-Pastonian by North-American paleontologists. This period had an important influence on the mitochondrial haplotype fragmentation of two Andean cats (*Leopardus pajeros* and *Leopardus jacobita*; Cossíos et al., 2009, 2012; Ruiz-García et al., 2013f) and we must recall that *lugens* is an Andean taxon. This period is within what the Argentinian paleontologists call Ensenadense (2–0.5 MYA). From about 1 to 0.7 MYA, the fauna of the Buenos Aires province was typical of the semi-arid Patagonia with a predominance of guanaco, Patagonian weasel (*Lestodelphys*) and the small ferret (*Lyncodon*). This could have favored the mitochondrial haplotype fragmentation of *lugens* in different Andean valleys of the current Colombia. Many of the populations of this taxon could be extremely small especially due to Andean forest fragmentation. This can generate elevated levels of gene drift inside each one of these populations (loss of gene diversity within these populations). However, taking all of these fragmented populations as a unique set drastically increases the gene diversity of this taxon. Although, in 2014, IUCN classified this taxon as critically endangered A3cd with a decreasing population trend, the levels of gene diversity are high.

The two *L. lagotricha* taxa (*cana* and *lagotricha*) which showed the lowest levels of gene diversity, were also the two taxa which showed the most recent mitochondrial haplotype diversification. *Cana* began its mitochondrial diversification around 0.5 MYA. This coincides with a period named by the Argentinian paleontologists as Bonaerense. This period was globally characterized by pulses of higher climatic stability in an environment warmer than previous periods. In this period there was an increase of the diversity in several mammal taxa (for instance, rodents, deer and the famous *Megatherium americanum*). In the Buenos Aires province, there were soils with a very diverse flora composition, which revealed an eco-systemic complexity (Forasiepi et al., 2007). This could be an important moment in the history of *cana* mitochondrial diversification. After *poepigii*, *cana* is probably the taxon which shows some geographical genetic structure among different rivers such as the Madeira, Tefé and Purús. It was classified by IUCN in 2014 as Endangered A2cd with a decreasing population trend.

Lagotricha was the taxon which showed the most recent mitochondrial diversification around 0.32 MYA. This coincides with the Mindel glacial period (named Elster, Kamasien I and Kansas depending on the area of earth area where it occurred) which began around 0.35 MYA. It was classified by IUCN in 2014 as Vulnerable A3cd and with a decreasing population trend.

The ancestors of *L. flavicauda* and *L. lagotricha* diverged around 2.4 MYA. This agrees quite well with the beginning of the Pleistocene (2.5 MYA) and the beginning of the first glacial period within the Pleistocene, 2.4 MYA. The first glacial periods in the beginning of the Pleistocene have a periodicity of 20,000 years. Another one million years later this periodicity was about every 100,000 years. Van der Hammen (1992) detected an overall decrease of $4 \pm 2^\circ\text{C}$ in South America and a decrease of 500–1000 mm in overall precipitation. At 2500 masl, the temperature was 10°C lower than today and a great portion of the Andean forests disappeared and transformed into an open dry savannah (paramo). This also coincides with the last formation phase of the Central Andes and a very dry ambient temperature in the Andean mountains. The entire Andean chain between Cajamarca and Huancavelica in Peru appeared by volcanism in this period. Additionally, there was an important marine introgression named Interensenadense. The lagoons in Huacho (northern Lima) and the bay in the Ventanilla beach in the Peruvian coast were formed in this period. The ancestors of *L. flavicauda* could have survived in the Eastern Peruvian forest refuge area (current Andean forests in the San Martín Department and other neighboring departments) (Haffer, 1969). In this area, there are also two other Peruvian endemic primates: *Aotus miconax* and *Callicebus oenanthe*. Thus, the

ancestors of *L. flavicauda* could have evolved independently from other *Lagothrix* taxa in this Pleistocene refuge since the beginning of the Pleistocene.

We detected a gene diversity of zero for the two mitochondrial genes of *L. flavicauda*. This is extremely bad news from a conservation point of view for this species. This agrees with the Critically Endangered A4c classification of this taxon provided by the IUCN—listing it as having less than 250 individuals in the wild (Nowak, 1999). Although, we only sequenced eight individuals of this species (we needed three expeditions to Peru, 2007–2010, to obtain these eight samples in the last expedition), these samples came from two (Colan Cordillera and upper Mayo River) of the three areas where this species was registered at its rediscovery in 1974. No exemplars could be sampled in the area of the Abiseo River National Park. Therefore, we do not know if animals from this third area could have different mitochondrial haplotypes from those of the two northern populations that we sampled. We have sequenced diverse mitochondrial genes in many Neotropical primate taxa in different genera (*Alouatta*, *Aotus*, *Ateles*, *Cebuella*, *Cebus*, *Pithecia*, *Saguinus*, *Saimiri*) and species and we have never found a Neotropical primate taxon without mitochondrial gene diversity. Thus, the situation of *L. flavicauda* is not only critical from a demographic situation, it is also critical from a genetics point of view. Additionally, no zoos or other institutions have some “ex situ” reproductive programs for this Peruvian endemic species. These “ex situ” programs, as well as “in situ” international and Peruvian programs are urgently called upon to try to save this species.

It would be interesting if the IUCN used information of gene diversity levels to help construct its classifications of species status.

4.3. Demographic changes in the different taxa of *L. lagotricha*

As we showed above, *poepigii* and *lagotricha* were the two taxa showing the clearest evidence of population expansion. The BSP analysis for *poepigii* showed a constant and effective increase of females, but this increase has been higher in the last 0.7 MY, which coincides with the ending of the Pre-Pastonian glacial period and the beginning of the Bonaerense, with a warmer temperature. In the case of *lagotricha*, there was a very strong population expansion around 0.3 MYA, coinciding with an inter-glacial period, with a hot climate (Hoxniense, 0.2–0.3 MYA). *Cana* suffered a slight increase in its female effective numbers around 0.1 MYA. This coincides with another inter-glacial period (Eemiense, 0.08–0.15 MYA), characterized by a hot climate, abundant precipitation and extensive swampy forests of *Aliso*, *Vallea* and *Weinmannia* (Van der Hammen, 1992). *Lugens* was the unique taxon (excluding *L. flavicauda*, of course) which did not show any clear evidence of population expansion throughout its natural history, but it did show a clear population declination in the last 25,000 years. Within the time period of 30,000–10,000 YA there were many episodes of extreme dry and cold conditions. For example, from 30,000 to 23,000 YA, there was a very cold period in Europe named Dryas I. Within this period, the Fuquene lagoon near current Bogotá (Colombia) became dry (29,000 YA; Van der Hammen, 1992). This is an area inside the *lugens* distribution. Related to this, MacNeish (1979) determined that the changes in soil acidity and pollen type within the Pikimachay Cove in Peru corresponded to an extreme cold spell 23,000 YA. Later, around 19,000–16,000 YA, the Last Glacial Maximum (LGM or Dryer II) occurred, with the most extensive distribution of snow and ice in the Central Andes in the last 200,000 years. Metivier (1998) estimated that 18,000 YA, the glacial extension in North and Central Andes was around 371,306 km², whereas currently it is around 3220 km² (only 1% of the LGM). These conditions probably affected the two

Andean *Lagothrix* taxa (*L. l. lugens* and *L. flavicauda*) considerably more than to the Amazon taxa (*poepigii*, *lagotricha* and *cana*).

Future studies on population genetics and phylogeny of *Lagothrix* should consider analyzing additional molecular markers, especially nuclear, MHC and Y chromosomal genes. It would also be worthwhile to increase the number of individuals analyzed within the range of *lugens* (Andean Colombia), in the possible areas of hybridization between *poepigii* and *cana* (eastern bank of the Juruá River and along the left bank of the upper Ucayali River to the lower Pachitea River; Brazil and Peru) and the zones of the Andean mountains in Southern Peru and Northern Bolivia (*tschudii*). We also strongly suggest for future researchers to determine if some degree of gene variability is present in the most southern area of the distribution of *L. flavicauda* (Abiseo River National Park, Peru) especially because it is one of the most endangered primates in the world.

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